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THE TERATOGENIC POTENTIAL OF CACODYLIC ACID IN THE RAT AND MOUSE

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ABSTRACT

Cacodylic acid, an organic arsenical herbicide, was administered to time-pregnant albino CD rats and CD-1 mice on days 7-16 of gestation. The compound was given by gastric intubation as a solution in distilled water. Rats received 0, 7.5, 15, 30, 40, 50, or 60 mg/kg/day in 0.2 ml/day intubation volume; mice received 0, 200, 400, or 600 mg/kg/day in 0.1 ml/day. Following maternal sacrifice on day 18 (mice) and 21 (rats), fetuses were weighed and fixed for skeletal and visceral examinations. Fetal and maternal toxicity was observed in both species. In the mouse, maternal toxicity was evident at the lowest dose, while teratogenic response was confined to cleft palate at 400 and 600 mg/kg/day. The effective maternal toxic dose in the rat was 40 mg/kg/day. In this species, incidence of irregular palatine rugae, i.e., ridges that were discontinuous and/or not lying in apposition at the palatal raphe, was significantly ($p < 0.001$) dose-related. The results suggest an "apparent no effect level" for this anomaly below 30 mg/kg/day.

INTRODUCTION

Arsenic is of ecological interest due to its ubiquitous nature and the biological activity of its compounds. In addition, large quantities of arsenicals are introduced into the environment from industrial and, especially, agricultural activities.[1]

Cacodylic acid (dimethylarsinic acid) is an organic arsenical contact herbicide capable of defoliating or dessicating a wide variety of plants. The uses for which cacodylic acid is currently registered in the U.S. include: postemergent weed control in non-crop areas; defoliation of cotton; directed application in non-bearing citrus orchards; lawn renovation; and crown kill of undesired trees. In addition, it is a registered substitute chemical for certain uses of 2,4,5-trichlorophenoxyacetic acid. Indications from the field are that the total volume of use of cacodylic acid in the U.S. is on the increase and that cotton defoliation represents the most rapidly growing use.[2]

A number of investigations have been conducted to determine the teratogenic effects of inorganic arsenic compounds. Administration of sodium arsenate by single intraperitoneal injection on one of gestational days 6-12 in the mouse[3] or days 7-12 in the rat[4] or by intravenous injection on gestational day 8 in the golden hamster[5] resulted in a spectrum of anomalies including exencephaly and rib malformations. Similar effects were noted in the mouse[6] treated with a single intraperitoneal injection of sodium arsenite on one of days 7-12 during gestation.

There is little published information on the teratogenic potential and reproductive effects of organic arsenicals. Intraperitoneal injections of disodium methanarsonate and sodium dimethylarsinate in pregnant mice produced a variety of fetal malformations similar to that associated with inorganic arsenic treatment.[7] In earlier experiments by the present authors,[8] cacodylic acid of 90%

purity administered by gastric intubation during the period of organogenesis produced fetal toxicity and teratogenicity in both the rat and mouse. Cleft palate was observed in the mouse, while micrognathia, small lung, and irregular palatine rugae were reported in the rat. Palatine rugae are the transverse ridges extending laterally on both sides of the raphe of the palate. In control fetuses, the two large anterior rugae appear as continuous, straight, parallel ridges that meet in apposition at the midline ridge of the palate. Aberrant rugae were defined as non-continuous or not lying in apposition at the palatine raphe (Figure 1).

The present study was designed to: (1) compare the fetotoxic and teratogenic potential of pure cacodylic acid with that of the compound used earlier, (2) determine the "apparent no effect level" for the occurrence of irregular palatine rugae in the rat, and (3) evaluate the occurrence of small lung and micrognathia (shortened mandible) in the rat as suggested in our previous study.[8]

MATERIALS AND METHODS

Time-pregnant random-bred albino CD rats and CD-1 mice were obtained from the Charles River Breeding Laboratories, Wilmington, Massachusetts. Day 1 of pregnancy was recorded upon demonstration of sperm plug (mouse) or sperm in the vaginal smear (rat). Rats and mice were housed 3 and 5 per cage, respectively, and were fed commercial lab chow and water ad libitum. Animal rooms were maintained at a constant temperature range (22-26°C) with controlled photoperiod (12 hr light).

FIG. 1. Palates of 21 day rat fetuses. Normal palate of control fetus on right. Arrow indicates principal area of malalignment. Note irregular palatine rugae of cacodylic acid treated fetuses center and left.

Cacodylic acid of 99+% purity was obtained from The Ansul Company, Marinette, Wisconsin. The compound was administered in distilled water vehicle by gastric intubation during the period of major organogenesis (days 7-16 of gestation). Doses were selected on the basis of studies conducted earlier,[8] and calculations for the preparation of dosing solutions were based on the average maternal weight on day 6 of pregnancy. Mice received doses of 0, 200, 400, or 600 mg/kg/day; rats, 0, 7.5, 15, 30, 40, 50, or 60 mg/kg/day. Concurrent controls received distilled water alone. Intubation volumes were 0.1 ml/day and 0.2 ml/day for mice and rats, respectively.

Mice were killed by decapitation on day 18 of pregnancy; rats on day 21. The liver and gravid uterus were removed and weighed. Maternal weight change was considered the overall weight gain during pregnancy less the gravid uterus weight. The number of dead and/or resorbed and live fetuses was recorded. Live fetuses were weighed as a litter and examined for gross abnormalities. Half the litter was then placed in Bouin's solution for necropsy. The remainder was placed in 65% ethyl alcohol and, after removal of the viscera and fat pad, was cleared in one percent KOH and stained with alizarin red S to facilitate subsequent skeletal examination. In order to determine the "apparent no effect level" for irregular palatine rugae, all rat fetuses from the 7.5 and 15 mg/kg/day doses were placed in Bouin's solution for necropsy.

Previous studies[8] suggested that cacodylic acid induced lung hypoplasia and micrognathia in the rat. Since visual identification

of these anomalies may be difficult, the lung/body weight ratio and jaw length were measured. At sacrifice, fetuses designated for alizarin preparation were weighed individually. Their lungs were then removed and weighed and a lung/body weight ratio calculated. Two methods were employed to quantitate micrognathia, one using fetuses fixed in Bouin's solution and the other using fetuses prepared in alizarin red S stain. In the first method, photographs (12X magnification) were made of the left side of the head of the Bouin's specimens. The photographs were used to measure the length of the mandibular and maxillary portions of the jaw. A ratio of the length of the lower jaw to the upper jaw was then calculated. In the second method, the left mandible was removed from the alizarin fetuses and its absolute length recorded to the nearest tenth of a millimeter.

Data was analyzed using the litter as the experimental unit; therefore, standard errors represent the variation of litter means. Dose-response relationships were determined using Jonckheere's test.[9] Comparisons between treated and control groups were made using the Mann-Whitney U test,[10] and the incidence of anomalies was analyzed by Fisher's Exact Test.[11]

RESULTS

Mouse

The data from the mouse study are summarized in Table 1.

At the highest dose (600 mg/kg/day), 59% maternal mortality occurred. A significant ($p < 0.001$) dose-related reduction in

TABLE 1

Results of Prenatal Administration of Cacodylic Acid to the Mouse

Dose (mg/kg/day)	0	200	400	600
Conception				
No. inseminated	30	32	31	32
% Mortality	0	0	3	59
No. pregnant (term)	17	18	22	12
Maternal				
Avg. wt. gain (g)	3.9 ± 0.4[a,e]	2.9 ± 0.4[f]	2.1 ± 0.4[g]	0.3 ± 0.5[h]
Avg. liver/body wt. (%)	6.7 ± 0.1	6.8 ± 0.1	6.9 ± 0.1	6.9 ± 0.2
Fetal				
Avg. no. implants	12.7 ± 0.6	12.2 ± 0.4	12.0 ± 0.5	12.6 ± 0.6
Avg. % mortality	13.0 ± 3.0[c]	16.5 ± 4.0	17.5 ± 4.4	39.8 ± 10.1[g]
Avg. fetal wt. (g)	0.98 ± 0.04[e]	0.96 ± 0.03	0.81 ± 0.03[g]	0.79 ± 0.04[g]
Avg. no. sternal ossification centers	5.3 ± 0.2	5.5 ± 0.2	4.7 ± 0.4	4.7 ± 0.6
Avg. no. caudal ossification centers	3.5 ± 0.3[e]	3.0 ± 0.4	2.0 ± 0.3[h]	2.2 ± 0.4[f]
Avg. % supernumerary ribs	30.6 ± 7.2[c]	33.6 ± 7.3	18.9 ± 5.6[f]	12.0 ± 5.6[f]
Avg. % irregular palatine rugae	0.0 ± 0.0	0.0 ± 0.0	4.8 ± 3.7	0.0 ± 0.0
Anomalies				
Cleft palate	0/0[b]	0/0	12/28[h]	7/21[h]

[a] Mean ± standard error of the mean.
[b] No. of litters/no. of fetuses.
[c] Significant dose-related response $p < 0.05$.
[d] Significant dose-related response $p < 0.01$.
[e] Significant dose-related response $p < 0.001$.
[f] Significantly different from control $p < 0.05$.
[g] Significantly different from control $p < 0.01$.
[h] Significantly different from control $p < 0.001$.

average maternal weight gain was noted, with the reduction significant ($p < 0.05$) at the lowest dose tested (200 mg/kg/day).

Fetal mortality was significantly ($p < 0.01$) elevated only at the highest dose administered. At doses greater than 200 mg/kg/day, significant retardations in fetal weight ($p < 0.01$) and caudal ossification centers ($p < 0.05$) occurred. The incidence of supernumerary ribs decreased significantly ($p < 0.05$) with dose.

TABLE 2

Results of Prenatal Administration

Dose (mg/kg/day)	0	7.5	15
Conception			
No. inseminated	40	21	21
% Mortality	0	0	0
No. pregnant (term)	36	17	18
Maternal			
Avg. wt. gain (g)	92.6 ± 4.4[a,d]	119.4 ± 4.5	115.6 ± 3.8
Avg. liver/body wt. (%)	4.8 ± 0.1	4.8 ± 0.1	5.1 ± 0.1
Fetal			
Avg. no. implants	10.2 ± 0.6	8.8 ± 0.7	10.8 ± 0.3
Avg. % mortality	7.4 ± 1.7[d]	13.2 ± 2.4	4.4 ± 1.8
Avg. fetal wt. (g)	4.33 ± 0.05[d]	4.60 ± 0.05	4.32 ± 0.06
Avg. no. sternal ossification centers	5.7 ± 0.1[d]	-	-
Avg. no. caudal ossification centers	4.9 ± 0.1[d]	-	-
Avg. % supernumerary ribs	28.1 ± 6.7[c]	-	-
Avg. % irregular palatine rugae	0.0 ± 0.0[d]	0.0 ± 0.0	2.1 ± 1.0
Lung/body wt.	3.4 ± 0.1	-	-
Anomalies			
Cleft palate	0/0[b]	0/0	0/0

[a] Mean ± standard error of the mean.
[b] No. of litters/no. of fetuses.
[c] Significant dose-related response $p < 0.05$.
[d] Significant dose-related response $p < 0.001$.
[e] Significantly different from control $p < 0.05$.
[f] Significantly different from control $p < 0.01$.
[g] Significantly different from control $p < 0.001$.

At the 400 mg/kg/day dose, cleft palate was observed in 57% of the litters with live fetuses, while 70% of the litters were affected at 600 mg/kg/day. No cleft palates were seen in the controls or in the 200 mg/kg/day dose g o. Irregular palatine rugae were noted in a total of four fetuses from two litters at the 400 mg/kg/day dose.

of Cacodylic Acid to the Rat

30	40	50	60
21	37	28	21
0	3	14	67
17	34	21	7
102.2 ± 3.8	75.0 ± 4.7[f]	61.9 ± 3.2[g]	48.4 ± 15.8[f]
4.8 ± 0.1	4.9 ± 0.1	4.8 ± 0.1	5.3 ± 0.2
10.2 ± 0.8	9.0 ± 0.7	8.7 ± 0.9	6.4 ± 2.3
8.0 ± 4.1	20.8 ± 5.2	32.9 ± 8.2[f]	65.4 ± 16.9[f]
4.34 ± 0.09	3.87 ± 0.12[g]	3.66 ± 0.14[g]	3.14 ± 0.27[g]
5.6 ± 0.2	5.3 ± 0.1[e]	5.0 ± 0.2[f]	4.1 ± 0.8[f]
4.7 ± 0.3	4.1 ± 0.2[g]	4.2 ± 0.3[e]	3.6 ± 0.2[f]
45.8 ± 17.6	11.1 ± 4.8[e]	15.2 ± 5.9	7.3 ± 7.3
14.6 ± 5.6[f]	65.6 ± 7.5[g]	86.2 ± 7.2[g]	72.3 ± 14.7[f]
3.0 ± 0.1	3.4 ± 0.1	3.4 ± 0.1	3.2 ± 0.3
0/0	0/0	0/0	0/0

Rat

The results of the rat study appear in Table 2.

Maternal mortality reached 67% at the highest dose tested (60 mg/kg/day). Maternal weight gain was significantly ($p < 0.01$) reduced at doses of 40 mg/kg/day and higher.

Significant elevations ($p < 0.01$) in fetal mortality occurred at 50 and 60 mg/kg/day, while fetal weight significantly decreased

($p < 0.001$) at 40 mg/kg/day and above. Sternal and caudal ossification centers were significantly ($p < 0.05$) retarded at doses of 40 mg/kg/day and greater. A significant ($p < 0.05$) dose-related decrease in the occurrence of supernumerary ribs was noted.

The incidence of irregular palatine rugae increased significantly ($p < 0.001$) with dose. Statistically significant ($p < 0.01$) levels of aberrant rugae occurred at the 30 mg/kg/day dose and higher, although this anomaly was observed as low as the 15 mg/kg/day dose. No palatine irregularities were noted in the controls or in the 7.5 mg/kg/day dose. No cleft palates were observed at any dose level.

Lung/body weight ratios were not affected by treatment. Quantitative analysis of lower jaw length demonstrated no significant differences between fetuses in the 50 mg/kg/day dose group (highest dose data available) and the controls. Measurements of the absolute lower jaw length taken on alizarin-stained fetuses from that dose averaged 0.81 cm ± 0.02, compared to 0.79 cm ± 0.02 in the controls.

DISCUSSION

Treatment of gravid CD-1 mice and CD rats with cacodylic acid during the period of organogenesis resulted in maternal and fetal toxicity. The effects reported here are similar to those described earlier.[8] In the present study, statistically significant maternal effects were evident in the mouse at all dose levels while the effective dose for the fetus appears to be 400 mg/kg/day. Teratogenic response in the mouse was confined to the occurrence of cleft

palate at the 400 and 600 mg/kg/day doses. The maternal effective dose in the rat appears to be 40 mg/kg/day, while the incidence of irregular palatine rugae in the fetus was significant at 30 mg/kg/day.

The occurrence of irregular palatine rugae in the present study confirmed our earlier findings.[8] The addition of lower doses in the current experiment indicated that this anomaly occurs in statistically significant numbers in dose groups as low as 30 mg/kg/day. The lower dose range also suggested that the "apparent no effect level" for the occurrence of aberrant rugae may be below 15 mg/kg/day, since isolated incidences of the defect were observed in this dose group, while none were seen at 7.5 mg/kg/day or in the controls. To our knowledge, with the exception of our earlier findings,[8] this abnormality has not been previously reported in the literature, although it has recently been shown that prenatal administration of phenytoin in rats also induces aberrant rugae.[12] A discriminatory and auxiliary masticatory function has been proposed for the palatine ridges of rats.[13] It has also been suggested that the rugae may play a role in food transport while the rat is eating.[14] However, the functional significance of aberrant rugae in the rat remains in question.

The occurrence of both small lung and micrognathia in the rat was suggested earlier.[8] At comparable doses in the current investigation, however, neither visual examination nor objective indices demonstrated any significant differences between the control and treated fetuses. This contrast in the findings of the two studies

may be due to differences in the purity of the compound and/or improved quantitation of the anomalies.

At the doses administered in the experiments reported here, cacodylic acid is toxic to both the rat and the mouse. Fetal effects in the mouse were observed at dose levels at which maternal toxicity was also noted, while in the rat, however, fetal effects occurred at doses below those at which maternal response to treatment was evident.

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